



ORIGINAL

PATENT

Our Docket: P-LG 4878

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Padgett et al.

Serial No. 10/066,390

Filed: February 1, 2002

For: A METHOD OF INCREASING
COMPLEMENTARITY IN A
HETERODUPLEX

Examiner: Unassigned

Group Art Unit: 1655

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PRELIMINARY AMENDMENT

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The Applicants submit the following amendment and
remarks.

I. AMENDMENT

Please cancel original claims 1 to 65, and add the
following new claims:

- - 66. An *in vitro* method of making linear sequence
variants from at least one heteroduplex polynucleotide where said
heteroduplex has at least two non-complementary nucleotide base
pairs, said method comprising:

- a. preparing at least one heteroduplex
polynucleotide;
- b. combining said heteroduplex polynucleotide with an
effective amount of CEL I, T4 DNA polymerase, and
T4 DNA ligase; and
- c. allowing sufficient time for the percentage of

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complementarity to increase, wherein one or more variants are made.

67. An *in vitro* method of making linear sequence variants from at least one heteroduplex polynucleotide wherein said heteroduplex has at least two non-complementary nucleotide base pairs, said method comprising:

- a. preparing at least one heteroduplex polynucleotide;
- b. combining said heteroduplex polynucleotide with an effective amount of an agent or agents with exonuclease activity, polymerase activity and strand cleavage activity; and
- c. allowing sufficient time for the percentage of complementarity to increase, wherein at least one or more variants are made.

68. The method of claim 67 wherein said agent having strand cleavage activity is added first, the agent having 3' to 5' exonuclease activity is added second, and the agent having polymerase activity is added third.

69. The method of claim 67 wherein said agents having exonuclease activity, polymerase activity, and strand cleavage activity are added concurrently.

70. The method of claim 67 in step (b) further comprising ligase activity.

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71. The method of claim 69 further comprising a step of,

(d) adding a ligase.

72. The method of claim 70 wherein said ligase is T4 DNA ligase, *E. coli* DNA ligase, or Taq DNA ligase.

73. The method of claim 67 wherein said agent with strand cleavage activity is an endonuclease enzyme.

74. The method of claim 67 wherein said agent with strand cleavage activity is selected from the group consisting of CEL I, T4 endonuclease VII, T7 endonuclease I, S1 nuclease, BAL-31 nuclease, FEN1, cleavase, pancreatic DNase I, SP nuclease, mung bean nuclease, and nuclease P1.

75. The method of claim 67 wherein said agent with strand cleavage activity is a chemical.

76. The method of claim 67 wherein said agent with strand cleavage activity is selected from the group consisting of potassium permanganate, tetraethylammonium acetate, sterically bulky photoactivatable DNA intercalators, $[\text{Rh}(\text{bpy})_2(\text{chrysi})]^{3+}$, osmium tetroxide with piperidine, and hydroxylamine with piperidine.

77. The method of claim 67 wherein said agent with strand cleavage activity is ionizing radiation, or kinetic radiation.

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78. The method of claim 67 wherein said agent with polymerase activity is T4 DNA polymerase or T7 DNA polymerase.

79. The method of claim 67 wherein said agent with both polymerase activity and 3' to 5' exonuclease activity is T4 DNA polymerase, T7 DNA polymerase, *E. coli* Pol 1, or Pfu DNA polymerase.

80. The method of claim 67 wherein said agent with both polymerase activity and 5' to 3' exonuclease activity is *E. coli* Pol 1.

81. The method of claim 67 wherein said effective amount of strand cleavage activity, and exonuclease activity/polymerase activity and ligase activity are provided by CEL I, T4 DNA polymerase, and T4 DNA ligase.

82. The method of claim 67 wherein said effective amount of strand cleavage activity, and exonuclease activity/polymerase activity and ligase activity are provided by CEL I, T7 DNA polymerase, and T4 DNA ligase.

83. The method of claim 67 wherein an effective amount of strand cleavage activity, and exonuclease activity/polymerase activity and ligase activity are provided by T4 endonuclease VII, T4 DNA polymerase, and T4 DNA ligase.

84. The method of claim 67 wherein complementarity within a heteroduplex is increased.

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85. The method of claim 67 wherein complementarity is complete yielding a homoduplex polynucleotide.

86. The method of claim 67 wherein diversity in a population of polynucleotides is increased. - -

REMARKS

The original claims have been cancelled, and replaced with claims to a particular aspect of the taught invention in the specification. The new claims are directed to an *in vitro* method of making linear sequence variants. For example, new claims 66 and 67 correspond to original claims 1 and 2, except that the new claims are directed linear sequence variants. New claim 68 is amended from original claim 7 to recite the order and types of activities each of the agents is providing. New claims 69 to 72 correspond to original claims 8 to 10, and 13 respectively. New claim 73 is amended from original claim 14 to recite an "endonuclease" enzyme. New claims 74 to 77 correspond to original claims 16 to 19. New claim 78 is amended from original claim 20 to include T7 DNA polymerase. New claims 79 to 83 correspond to original claims 22 to 26, respectively. New claims 84 to 86 are new dependent claims to original claim 2, i.e., new claim 67. Therefore, new claims 66 to 86 do not constitute new subject matter.

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CONCLUSION

In light of the Amendments and Remarks herein,
Applicants submit that the claims are now in condition for
allowance and respectfully request a notice to this effect.
Should the Examiner have any questions, he/she is invited to call
Cathryn Campbell or the undersigned attorney.

Respectfully submitted,

July 25, 2002
Date

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